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COVID-19 targets human adrenal glands

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Abstract: Diabetes is one of the most important comorbidities linked to the severity of all three known human pathogenic coronavirus infections, including severe acute respiratory syndrome coronavirus 2. Patients with diabetes have an increased risk of severe complications including Adult Respiratory Distress Syndrome and multi-organ failure. Depending on the global region, 20–50% of patients in the coronavirus disease 2019 (COVID-19) pandemic had diabetes. Given the importance of the link between COVID-19 and diabetes, we have formed an international panel of experts in the field of diabetes and endocrinology to provide some guidance and practical recommendations for the management of diabetes during the pandemic. We aim to briefly provide insight into potential mechanistic links between the novel coronavirus infection and diabetes, present practical management recommendations, and elaborate on the differential needs of several patient groups.

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COVID-19 targets human adrenal glands

COVID-19 develops due to infection with SARS-CoV-2, which particularly in elderly with certain comorbidities (eg, metabolic syndrome)¹ can cause severe pneumonia and acute respiratory distress syndrome. Some patients with severe COVID-19 will develop a life-threatening sepsis with its typical manifestations including disseminated intravascular coagulation and multiorgan dysfunction.² Latest evidence suggests that even early treatment with inhaled steroids such as budesonide might prevent clinical deterioration in patients with COVID-19.³ This evidence underlines the potentially important role for adrenal steroids in coping with COVID-19.

The adrenal gland is an effector organ of the hypothalamic-pituitary-adrenal axis and the main source of glucocorticoids, which are critical to manage and to survive sepsis. Therefore, patients with pre-existing adrenal insufficiency are advised to double their doses of glucocorticoid supplementation after developing moderate to more severe forms of COVID-19.⁴

Adrenal glands are vulnerable to sepsis-induced organ damage and their high vascularisation and blood supply makes them particularly susceptible to endothelial dysfunction and haemorrhage. Accordingly, adrenal endothelial damage, bilateral haemorrhages, and infarctions have been already reported in patients with COVID-19.⁵ Adrenal glands contain the highest concentration of antioxidants to compensate enhanced generation of reactive oxygen species, side products of steroidogenesis, which together with elevated intra-adrenal inflammation can contribute to adrenocortical cell death.⁶ Furthermore, sepsis-associated critical illness-related corticosteroid insufficiency, which describes coexistence of the hypothalamic-pituitary-adrenal dysfunction,

reduced cortisol metabolism, and tissue resistance to glucocorticoids, was reported in critically ill patients with COVID-19.⁷ Low cortisol and adrenocorticotrophic hormone (ACTH) responses during acute phase of infections consistent with critical illness-related corticosteroid insufficiency diagnosis (random plasma cortisol level lower than 10 µg/dL) were reported in one study with patients suffering from mild to moderate COVID-19 manifestations.⁸ It is however possible those other factors triggered by COVID-19 such as hypothalamic or pituitary damage, adrenal infarcts, or previously undiagnosed conditions, such as antiphospholipid syndrome, might be responsible for reduced function of adrenal glands. However, contrary to this observation, a study with patients with moderate to severe COVID-19 revealed a very high cortisol response with values exceeding 744 nmol/L, which were positively correlated with severity of disease.⁹ In this clinical study,⁹ highly elevated cortisol concentrations showed an adequate adrenal cortisol production possibly reflecting the elevated stress level of those severely affected patients.⁹ However, since ACTH measurements were not done, it is impossible to verify whether high concentrations of cortisol in those patients resulted from an increment of cortisol, or were confounded by reduced glucocorticoid metabolism.⁹

A critical and yet unsolved major question is whether SARS-CoV-2 infection can contribute either directly or indirectly to adrenal gland dysfunction observed in some patients with COVID-19 or contribute to the slow recovery of some patients with long COVID.

We performed a comprehensive histopathological examination of adrenal tissue sections from autopsies of patients that died due to COVID-19 (40 cases), collected from three different pathology centres in Regensburg, Dresden, and Zurich

(appendix pp 1–3). We observed evidence of cellular damage and frequently small vessel vasculitis (endotheliitis) in the periadrenal fat tissue (six cases with low and 13 cases with high density; appendix p 10) and much milder occurrence in adrenal parenchyma (ten cases with low and one case with moderate score; appendix p 10), but no evidence of thrombi formation was found (appendix p 10). Endotheliitis has been scored according to a semi-quantitative immunohistochemistry analysis as described in the appendix (p 4). Additionally, in the majority of cases (38 cases), we noticed enhanced perivascular lymphoplasmacellular infiltration of different density and sporadically a mild extravasation of erythrocytes (appendix p 10). However, no evidence of widespread haemorrhages and degradation of adrenocortical cells were found, which is consistent with histological findings reported previously.⁵ In another autopsy study analysing adrenal glands of patients with COVID-19, additional signs of acute fibrinoid necrosis of small vessels in adrenal parenchyma, subendothelial vacuolisation and apoptotic debris were found.⁵

Adrenal gland is frequently targeted by bacteria and viruses, including SARS-CoV,¹⁰ which was responsible for the 2002–04 outbreak of SARS in Asia. Considering that SARS-CoV-2 shares cellular receptors with SARS-CoV, including angiotensin-converting enzyme 2 and transmembrane protease serine subtype 2, its tropism to the adrenal gland is therefore conceivable.

To investigate whether adrenal vascular cells and possibly steroid-producing cells are direct targets of SARS-CoV-2, we examined SARS-CoV-2 presence in adrenal gland tissues obtained from the 40 patients with COVID-19 (appendix pp 1–3). Adrenal tissues from patients who died before the COVID-19 pandemic were used as negative controls to validate antibody



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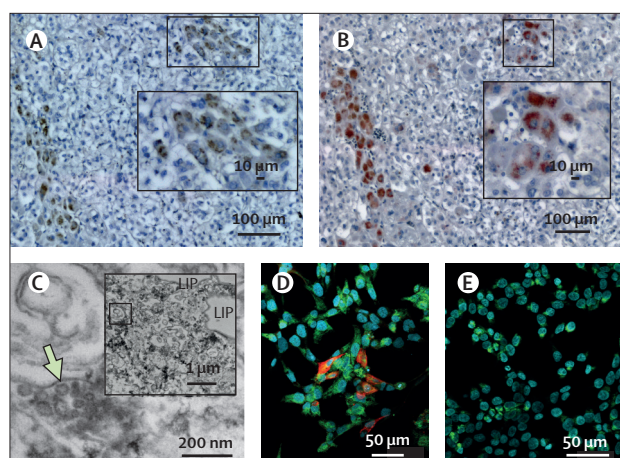


Figure: Detection of SARS-CoV-2 in human adrenal gland from a patient who died due to COVID-19

(A) Detection of SARS-CoV-2 RNA by in situ hybridization (ISH; brown DAB staining) and (B) its spike protein by immunohistochemistry (red AEC staining) was found in the same region of two serial 1 µm-thick tissue sections of the adrenal gland. Inserts depict the same regions but were captured at lower magnification showing the chosen area of virus positivity (white squares). Scattered expression of either mRNA or protein can be found in the inner parts of the adrenal gland cortex close to the medulla. Scale bars in pictures from panel A and B represent 100 µm (10 µm in the insert image). (C) Ultrastructural examination of a SARS-CoV-2 triple positive (ISH, immunohistochemistry, and RT-qPCR) adrenal tissue showing numerous viral-like particles in liposome (LIP)-rich adrenocortical cells. A scale bar represents 200 nm. A low magnification picture shown in the insert depicts the region of interest indicated by a green arrow in the enlarged picture. Scale bar in insert indicates 1 µm distance. (D) A positive immunofluorescent red signal (CY3) indicating the expression of spike protein in SARS-CoV-2 infected human adrenocortical cells. (E) Lack of positive signal (CY3) in mock-infected control cells. Adrenocortical cells were additionally stained with an antibody against side-chain cleavage enzyme (CYP11A1), which is a steroidogenic marker (green signal; CY5). Nuclei were counterstained with Hoechst 33342 dye (blue signal). Scale bars in pictures from panel D and E represent 50 µm.

specificity. Using a monoclonal antibody (clone 1A9; appendix p 11), we detected SARS-CoV-2 spike protein in adrenocortical cells in 18 (45%) of 40 adrenal gland tissues (figure B; appendix p 12). In the same number of adrenal tissues (18 [45%] of 40), we have detected SARS-CoV-2 mRNA using in situ hybridisation (ISH; figure A; appendix p 12). The concordance rate between immunohistochemistry and ISH methods was 90% (36/40). Scattered and rather focal expression pattern of SARS-CoV-2 spike protein was found in the adrenal cortex (figure A and B; appendix p 12). In addition, SARS-CoV-2 expression was confirmed in 15 out of 30 adrenal gland tissues of patients with COVID-19 by multiplex RT-qPCR (appendix pp 6–7). The concordance between ISH, immunohistochemistry, and

RT-qPCR techniques for SARS-CoV-2 positivity was only 23%, which is a technical limitation of our study possibly reflecting the low number of virus-positive cells. However, when considering triple-negative samples, an overall 53% consensus was found (appendix pp 7–8).

Finally, to confirm the identity of infected cells, we have performed an ultrastructural analysis of adrenal tissue from a triple-positive patient case (by immunohistochemistry, ISH, and RT-qPCR), and found numerous SARS-CoV-2 virus-like particles in cells enriched with liposomes, which are typical markers of adrenocortical cells (figure C). The cortical identity of SARS-CoV-2 spike positive cells was also shown using serial tissue sections, demarcating regions with double positivity for viral protein and StAR RNA (appendix p 12). Furthermore, susceptibility of adrenocortical cells to SARS-CoV-2 infection was confirmed by in-vitro experiments (appendix p 7) showing detection of viral spike protein in adrenocortical carcinoma cells (NCI-H295R) cultured in a medium containing SARS-CoV-2 (figure D), and its absence in mock-treated control cells (figure E). We showed an uptake of viral particles in the adrenocortical cells, by ISH, immunohistochemistry, RT-qPCR and electron microscopy (figure A–C). Mechanistically, an uptake of SARS-CoV-2 like particles might involve expression of ACE2 in vascular cells (appendix p 13) and perhaps of the shorter isoform of ACE2 together with TMPRSS2 and other known or currently unknown virus-entry facilitating factors in adrenocortical cells (appendix p 13). An example of such factor is scavenger receptor type 1, which is highly expressed in adrenocortical cells.¹¹

Several forms of regulated cell necrosis were implicated in sepsis-mediated adrenal gland damage.⁶ One of the prime examples of regulated necrosis triggered by sepsis-associated tissue inflammation is necroptosis.

The necrotic process is characterised by loss of membrane integrity and release of danger-associated molecular patterns, which further promote tissue inflammation (necroinflammation) involving enhanced activation of the complement system and related activation of neutrophils. Whether necroptosis might be involved in COVID-19-associated adrenal damage is currently unknown. In our study, we showed prominent expression of phospho Mixed Lineage Kinase Domain Like Pseudokinase (pMLKL) indicating necroptosis activation in adrenomedullary cells (appendix p 14) in adrenal glands of COVID-19 patients. However, since we have also observed pMLKL expression in adrenal glands obtained from autopsies done before the COVID-19 pandemic (controls), necroptosis activation in medullary cells might be a rather frequent and SARS-CoV-2 independent event. However, contrary to the adrenal medulla, pMLKL positivity in the adrenal cortex was only found in virus-positive regions (appendix p 14). This finding suggests that SARS-CoV-2 infection might have directly triggered activation of necroptosis in infected cells in the adrenal cortex, whereas pMLKL expression in the adrenal medulla seems rather an indirect consequence of systemic inflammation.

In summary, in our study of 40 patients who died from COVID-19, we did not observe widespread degradation of human adrenals that might lead to manifestation of the adrenal crisis. However, our study shows that the adrenal gland is a prominent target for the viral infection and ensuing cellular damage, which could trigger a predisposition for adrenal dysfunction. Whether those changes directly contribute to adrenal insufficiency seen in some patients with COVID-19 or lead to its complications (such as long COVID) remains unclear. Large multicentre clinical studies should address this question.

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